

14 May 2009

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**CONTROL THE GROWTH OF INTRACELLULAR BACTERIA:** *"Thus, our results indicate functional similarities between immune T cells residing in spleens, livers, and lungs of LVS-immune mice."*

### **8. IMMUNE EXHAUSTION DRIVEN BY ANTIGEN IN CHRONIC VIRAL INFECTION:**

*"The T cells, or white blood cells, fighting a chronic infection eventually wear out."*

# **CB Daily Report**

## **Chem-Bio News**

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**DETECTION OF ANTHRAX TOXIN BY AN ULTRASENSITIVE IMMUNOASSAY USING EUROPIUM NANOPARTICLES**

Immunotherapy Weekly

May 6, 2009

"We developed a europium nanoparticle-based immunoassay (ENIA) for the sensitive detection of anthrax protective antigen (PA). The ENIA exhibited a linear dose-dependent pattern within the detection range of 0.01 to 100 ng/ml and was approximately 100-fold more sensitive than enzyme-linked immunosorbent assay (ELISA)."

"False-positive results were not observed with serum samples from healthy adults, mouse plasma without PA, or plasma samples collected from mice injected with anthrax lethal factor or edema factor alone. For the detection of plasma samples spiked with PA, the detection sensitivities for ENIA and ELISA were 100% (11/11 samples) and 36.4% (4/11 samples), respectively. The assay exhibited a linear but qualitative correlation between the PA injected and the PA detected in murine blood ( $r = 0.97731$ ;  $P < 0.0001$ ). Anthrax PA was also detected in the circulation of mice infected with spores from a toxigenic Sterne-like strain of *Bacillus anthracis*, but only in the later stages of infection."

"These results indicate that the universal labeling technology based on europium nanoparticles and its application may provide a rapid and sensitive testing platform for clinical diagnosis and laboratory research."

The full article can be found at: (S.X. Tang, et. al., "Detection of Anthrax Toxin by an Ultrasensitive Immunoassay Using Europium Nanoparticles". *Clinical and Vaccine Immunology*, 2009;16(3):408-413). Link not available.

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## **COMPARISON OF HISTOPATHOLOGICAL FEATURES OF VIBRIO CHOLERAE O1 EL TOR AND O139 BENGAL INFECTIONS IN RABBIT INTESTINAL MUCOSA**

Gastroenterology Week

May 11, 2009

"There are > 100 serovars of *V. cholerae*, but the O1 and O139 serovars are the main causative agents of cholera. The present study aimed to compare the severity of intestinal mucosal infection caused by O1 El Tor and O139 *V. cholerae* in a rabbit ileal loop model. The results showed that although the fluid accumulation was similar in the loops inoculated with O1 and O139 *V. cholerae*, the presence of blood was detected only in the loops inoculated with the O139 serovar. Serosal hemorrhage was confirmed by histopathological examination and the loops inoculated with O139 showed massive destruction of villi and loss of intestinal glands. The submucosa and muscularis mucosa of the ileum showed the presence of edema with congested blood vessels, while severe hemorrhage was seen in the muscularis propria layer. The loops inoculated with O1 El Tor showed only minimal damage, with intact intestinal villi and glands. Diffuse colonies of the O139 serovar were seen to have infiltrated deep into the submucosal layer of the intestine. Although the infection caused by the O1 serovar was focal and invasive, it was more superficial than that due to O139, and involved only the villi. These observations were confirmed by immunostaining with O1 and O139 *V. cholerae*-specific monoclonal antibodies. The peroxidase reaction demonstrated involvement of tissues down to the submucosal layer in O139 *V. cholerae* infection, while in O1 El Tor infection, the reaction was confined mainly to the villi, and was greatly reduced in the submucosal region."

"This is the first reported study to clearly demonstrate the histopathological differences between infections caused by the O139 Bengal and O1 El Tor pathogenic serovars of *V. cholerae*."

The full article can be found at: (A. Amin, et. al., "Comparison of histopathological features of *Vibrio cholerae* O1 El Tor and O139 Bengal infections in rabbit intestinal mucosa". *Histology and Histopathology*, 2009;24(5):559-565). Link not available.

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## **ANTHRAX EDEMA TOXIN INDUCES MATURATION OF DENDRITIC CELLS AND ENHANCES**

## **CHEMOTAXIS TOWARDS MACROPHAGE INFLAMMATORY PROTEIN 3BETA**

Medical Letter on the CDC & FDA

May 10, 2009

"Bacillus anthracis secretes two bipartite toxins, edema toxin (ET) and lethal toxin (LT), which impair immune responses and contribute directly to the pathology associated with the disease anthrax. Edema factor, the catalytic subunit of ET, is an adenylate cyclase that impairs host defenses by raising cellular cyclic AMP (cAMP) levels."

"Synthetic cAMP analogues and compounds that raise intracellular cAMP levels lead to phenotypic and functional changes in dendritic cells (DCs). Here, we demonstrate that ET induces a maturation state in human monocyte-derived DCs (MDDCs) similar to that induced by lipopolysaccharide (LPS). ET treatment results in downregulation of DC-SIGN, a marker of immature DCs, and upregulation of DC maturation markers CD83 and CD86. Maturation of DCs by ET is accompanied by an increased ability to migrate toward the lymph node-homing chemokine macrophage inflammatory protein 3beta, like LPS-matured DCs. Interestingly, cotreating with LT differentially affects the ET-induced maturation of MDDCs while not inhibiting ET-induced migration."

"These findings reveal a mechanism by which ET impairs normal innate immune function and may explain the reported adjuvant effect of ET."

The full article can be found at: (F.J. Maldonado-Arocho, et. al., "Anthrax edema toxin induces maturation of dendritic cells and enhances chemotaxis towards macrophage inflammatory protein 3beta". Infection and Immunity, 2009;77(5):2036-42). Link not available.

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## **MOLECULAR ANALYSIS OF THE INTERACTION OF ANTHRAX ADENYLYL CYCLASE TOXIN, EDEMA FACTOR, WITH 2'(3')-O-(N-(METHYL)ANTHRANILOYL)-SUBSTITUTED PURINE AND PYRIMIDINE NUCLEOTIDES**

Medical Letter on the CDC & FDA

May 3, 2009

"Bacillus anthracis causes anthrax disease and exerts its deleterious effects by the release of three exotoxins: lethal factor, protective antigen, and edema factor (EF), a highly active calmodulin-dependent adenylyl cyclase (AC). However, conventional antibiotic treatment is ineffective against either toxemia or antibiotic-resistant strains."

"Thus, more effective drugs for anthrax treatment are needed. Previous studies from our laboratory showed that mammalian membranous AC (mAC) exhibits broad specificity for purine and pyrimidine nucleotides (Mol Pharmacol 70:878-886, 2006). Here, we investigated structural requirements for EF inhibition by natural purine and pyrimidine nucleotides and nucleotides modified with N-methylanthraniloyl (MANT)- or anthraniloyl groups at the 2'(3')-O ribosyl position. MANT-CTP was the most potent EF inhibitor (K<sub>i</sub>, 100 nM) among 16 compounds studied. MANT-nucleotides inhibited EF competitively. Activation of EF by calmodulin resulted in effective fluorescence resonance energy transfer (FRET) from tryptophan and tyrosine residues located in the vicinity of the catalytic site to MANT-ATP, but FRET to MANT-CTP was only small. Mutagenesis studies revealed that Phe586 is crucial for FRET to MANT-ATP and MANT-CTP and that the mutations N583Q, K353A, and K353R differentially alter the inhibitory potencies of MANT-ATP and MANT-CTP. Docking approaches relying on crystal structures of EF indicate similar binding modes of the MANT nucleotides with subtle differences in the region of the nucleobases."

"Like mAC, EF accommodates both purine and pyrimidine nucleotides. The unique preference of EF for the base cytosine offers an excellent starting point for the development of potent and selective EF inhibitors."

The full article can be found at: (H.M. Taha, et. al., "Molecular Analysis of the Interaction of Anthrax Adenylyl Cyclase Toxin, Edema Factor, with 2'(3')-O-(N-(Methyl)anthraniloyl)-Substituted Purine and

Pyrimidine Nucleotides". *Molecular Pharmacology*, 2009;75(3):693-703). Link not available.

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## **CALIBRATION OF THE MODIFIED ELECTRICAL LOW-PRESSURE IMPACTOR (ELPI) FOR USE WITH PRESSURIZED PHARMACEUTICAL AEROSOLS**

Drug Week

May 8, 2009

"The modified Electrical Low Pressure Impactor (ELPI) is currently being used in several laboratories to determine inherent electrostatic charge of pharmaceutical aerosols as a function of their particle size. However, the ELPI appears to underestimate the aerodynamic particle size distributions (aPSDs) of pressurized metered dose inhalers (pMDIs), casting doubt upon the manufacturer's calibration."

"In the present study, four commercially available pMDIs with a range of aPSDs were used to recalibrate cutoff diameters (d50s) of the ELPI stages using a reference ACI. Particle size analyses were performed in a mensurated ACI and a calibrated modified ELPI (n = 5); stage coating was employed in both instruments. The ACI data were fitted to a lognormal cumulative distribution function by nonlinear regression analysis. Best estimates for mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) for each pMDI were obtained and used in combination with impaction results from the modified ELPI to determine new d50s for each of the ELPI stages by numerical methods. Ventolin HFA (R) was employed to validate the new ELPI d50 values. The curve-fitting procedure produced excellent fits of the ACI data for all the calibration pMDIs, which were well modeled as mono-modal and log-normally distributed. The mean d50s obtained following recalibration of the modified ELPI were found to deviate increasingly from the manufacturer-supplied values as aerodynamic diameter decreased. Ventolin HFA's MMAD determined using the modified ELPI with the manufacturer-supplied d50s was 2.06 +/- 0.08  $\mu$  m. The MMAD calculated using the recalibrated d50s was 2.63 +/- 0.09  $\mu$  m, which was statistically indistinguishable (p = 0.0852) from that determined for Ventolin HFA using the ACI (2.73 +/- 0.09  $\mu$  m)."

"In the absence of a comprehensive recalibration of the ELPI using monodisperse aerosols, the mean d50s for stages 4-12 of ELPI reported offer a practical way of analyzing the aPSD of pharmaceutical aerosols based on the collection and chemical analysis of ELPI deposition data."

The full article can be found at: (R. Kotian, et. al., "Calibration of the Modified Electrical Low-Pressure Impactor (ELPI) for Use with Pressurized Pharmaceutical Aerosols". *Journal of Aerosol Medicine and Pulmonary Drug Delivery*, 2009;22(1):55-65). Link not available.

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## **DIFFERENTIAL EFFECTS OF STAPHYLOCOCCAL ENTEROTOXIN B-MEDIATED IMMUNE ACTIVATION ON INTESTINAL DEFENSINS**

Drug Week

May 8, 2009

"In the small intestine members of both the alpha-defensin (DEFA5 and DEFA6) and beta-defensin (DEFB1 and DEFB2) family contribute to the anti-microbial barrier against infection. The aim of this study was to determine whether Staphylococcal enterotoxin B (SEB)-mediated immune activation and proinflammatory cytokines play a role in the regulation of intestinal defensin expression."

"Defensin mRNA and peptide secretion was studied after ex vivo tissue culture of duodenal biopsies over 24 h. Immune (T cell and macrophage) activation was induced by SEB, and in separate experiments exogenous proinflammatory cytokines were added individually. Defensin mRNA levels were quantified by reverse transcription-polymerase chain reaction, and peptide release into culture supernatants was quantified by immuno dot blot or enzyme-linked immunosorbent assay. Increasing concentrations of SEB down-regulated DEFA5, DEFA6 and DEFB1 mRNA in a dose-dependent manner but increased DEFB2 simultaneously. The down-regulation of alpha-defensins was reversed by

dexamethasone. DEFA5 and DEFB2 peptide secretion levels were altered in parallel with mRNA. Interferon-gamma and interleukin (IL)-1beta exhibited a dose-dependent down-regulation of alpha-defensin mRNA, IL-6 significantly down-regulated only DEFA6; in contrast, tumor necrosis factor-alpha and IL-4 had no significant effect. Immune cell activation and proinflammatory cytokines down-regulated the constitutively expressed DEFA5, DEFA6 and DEFB1 defensins, and up-regulated DEFB2 in intact human intestinal tissue explants in short-term culture."

"The effect of local immune activation on innate defence may explain the reduced alpha-defensin expression noted in inflammatory T cell-mediated enteropathies."

The full article can be found at: (W. Dhaliwal, et. al., "Differential effects of Staphylococcal enterotoxin B-mediated immune activation on intestinal defensins". *Clinical & Experimental Immunology*, 2009;156(2):263-70). Link not available.

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## **T CELLS FROM LUNGS AND LIVERS OF FRANCISELLA TULARENSIS-IMMUNE MICE CONTROL THE GROWTH OF INTRACELLULAR BACTERIA**

Vaccine Weekly

May 6, 2009

"Parenteral and respiratory vaccinations with the intracellular bacterium *Francisella tularensis* have been studied using the live vaccine strain (LVS) in a mouse model, and spleen cells from immune mice are often used for immunological studies. However, mechanisms of host immunological responses may be different in nonlymphoid organs that are important sites of infection, such as lung and liver."

"Using parenteral (intradermal) or respiratory (cloud aerosol) vaccination, here we examine the functions of resulting LVS-immune liver or lung cells, respectively. Surprisingly, LVS was considerably more virulent when administered by cloud aerosol than by intranasal instillation, suggesting method-dependent differences in initial localization and/or dissemination patterns. Only low doses were sublethal, and resolution of sublethal cloud aerosol infection was dependent on gamma interferon (IFN-gamma), tumor necrosis factor alpha, and inducible nitric oxide synthase. Nonetheless, survival of cloud aerosol or parenteral infection resulted in the development of a protective immune response against lethal LVS intraperitoneal or aerosol challenge, reflecting development of systemic secondary immunity in both cases. Such immunity was further detected by directly examining the functions of LVS-immune lung or liver lymphocytes in vitro. Lung lymphocytes primed by respiratory infection, as well as liver lymphocytes primed by parenteral infection, clearly controlled in vitro intracellular bacterial growth primarily via mechanisms that were not dependent on IFN-gamma activity."

"Thus, our results indicate functional similarities between immune T cells residing in spleens, livers, and lungs of LVS-immune mice."

The full article can be found at: (C.M. Collazo, et. al., "T cells from lungs and livers of *Francisella tularensis*-immune mice control the growth of intracellular bacteria". *Infection and Immunity*, 2009;77(5):2010-21). Link not available.

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## **IMMUNE EXHAUSTION DRIVEN BY ANTIGEN IN CHRONIC VIRAL INFECTION**

Infection Control Today Magazine

May 13, 2009

"The T cells, or white blood cells, fighting a chronic infection eventually wear out.

Researchers at Emory Vaccine Center have demonstrated that exhaustion is driven by how the immune system detects infecting viruses.

To recognize the presence of a viral infection, T cells must be presented with bits of viral protein in a molecular frame supplied by other cells in the body -- called MHC (major histocompatibility complex) class I molecules.

In mice infected by lymphocytic choriomeningitis virus (LCMV), T cells became more or less exhausted depending on how much properly framed viral protein was available.

Insights from the research could guide efforts to revive the immune system in people with chronic viral infections. The results are published online this week in the Proceedings of the National Academy of Sciences.”

The full article can be found at: <http://www.infectioncontroltoday.com/hotnews/immune-exhaustion-viral-infection.html>

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